Infection of *Stylosanthes guianensis* and *S. scabra* by *Colletotrichum gloeosporioides*

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ABSTRACT

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Penetration and postpenetration development were investigated quantitatively and qualitatively by light and scanning electron microscopy. Penetration commenced by 24 hr after inoculation on most interactions and continued until at least 96 hr on some. The overall level of penetration was very low (<9%). After penetration, a vesicle formed within the epidermal cell beneath the appressorium. In compatible interactions, this cell plus two to four adjacent cells were usually filled with hyphae before the fungus spread intra- and intercellularly into the mesophyll. "Runner" hyphae emerged from the epidermal cells to grow subcuticularly or, more often, superficially, initiating new infections without the formation of appressoria. In incompatible interactions, colonies ceased growth, increased in size very slowly or apparently decreased in size. Most infections were restricted to one cell. No "runner" hyphae were observed. Host cell response to infection varied from the deposition of apposition layers on the inner surface of the epidermal cell wall or the aggregation of granular cytoplasm beneath an appressorium to browning of the cell wall and contents.

Stylosanthes spp. are among the most important pasture legumes in tropical and subtropical areas (2). Unfortunately, their usefulness is seriously threatened by the anthracnose diseases caused by the fungus, *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. (5). Two types of anthracnose disease have been reported (3). Symptoms of the type A form of the disease are lesions, 1–4 mm in diameter, light in the center, and surrounded by a dark margin. The type B form causes a general necrosis of the terminal shoots extending for several centimeters down the stem. Type B symptoms have only been observed in *Stylosanthes guianensis* (Aubl.) Sw. Pathogenic specialization has been reported for both forms of the disease (3,4).

Preliminary quantitative whole leaf studies showed that the infection process was similar for the two forms of the disease, but that the rate of development differed (7). Appressoria formed and became melanized more rapidly in type A isolates than in type B isolates. Penetration from appressoria of both types occurred by 12 hr after inoculation, at which time, subcuticular infection hyphae were also present. Inter- and intracellular growth was observed at 24 hr. Mesophyll cells were particularly susceptible to fungal invasion. Acervuli of type A isolates erupted 48 hr earlier than acervuli of type B isolates.

Type A-*Stylosanthes scabra* Vog. interactions have been more intensively investigated (16). Penetration occurred only from melanized appressoria and resulted in the formation of a pyriform to spherical vesicle within the epidermal cell beneath the appressorium. The cells of the stomatal complex were penetrated more frequently than other epidermal cells. In compatible interactions, up to six epidermal cells had been invaded by 48 hr after inoculation, but no cell degradation was evident. Hyphae were present in the mesophyll by 72 hr and both epidermal and mesophyll cells had degenerated.

Quantitative studies of the penetration and postpenetration phases of infection in compatible and incompatible type B-*S. guianensis* interactions have not been reported previously. Vinijsanun et al (17) reported that in the compatible interaction between a type B isolate and the highly susceptible *S. guianensis* 'Endeavour', the infection peg enlarged within the cuticle to produce a swelling (vesicle or knot) by 24 hr after inoculation. By 72 hr the host's epidermal cell wall had been penetrated and subcuticular, inter-, and intracellular growth had occurred. However, palisade mesophyll cells had not collapsed. Infection by a type B isolate of *C. gloeosporioides* on cultivar Endeavour occurred more frequently through cells of the stomatal complex than through epidermal cells (10). Following penetration, one to three epidermal cells were filled with hyphae before rapid expansion of the colony and the formation of subcuticular hyphae.

This paper reports quantitative whole leaf studies of the penetration and postpenetration phases of infection of six genetically defined cultivars or accessions of *S. guianensis* by three type B pathotypes of *C. gloeosporioides*. These studies were made to obtain more detailed information about the infection process and the host response to infection in compatible and incompatible interactions. Subsequently, qualitative studies of resin-embedded sections of a compatible type B infection were made to determine the location of the infection vesicle and the secondary infection hyphae. Further qualitative comparisons of compatible type A and type B infections on whole leaves by light and scanning electron microscopy were made to confirm suspected differences in the mode of infection between the two types of the disease. These qualitative studies are also reported here.

MATERIALS AND METHODS

Quantitative studies of infection of *S. guianensis* by type B pathotypes of *C. gloeosporioides*. *Plant material.* Two commercial cultivars of *S. guianensis*, Endeavour and Graham, were used together with the Commonwealth Plant Introduction accessions 18750, 34911, 79637, and 79639, supplied by D. F. Cameron, Division of Tropical Crops and Pastures, CSIRO. Resistance is conditioned by dominant genes. The single genes conditioning resistance in 18750 and 34911 appear to be closely linked or at

the same locus. Graham and 79639 probably share a resistance gene but at a different locus to that present in 18750 and 34911. Accession 79637 appears to carry two dominant genes for resistance, one of which may be allelic with the 34911 gene (Cameron and Irwin, *unpublished data*).

Plants were grown in the glasshouse in 15-cm-diameter plastic pots in steam-air pasteurized peat/sand (1:1, v:v) mix with the necessary nutrients added.

Fungal material. Monoconidial isolates of three type B pathotypes of *C. gloeosporioides* were used. Pathotype 1 (accession 21808) was isolated from *S. guianensis* cultivar Schofield from Julatten, North Queensland, pathotype 2 (accession 22351) from *S. guianensis* line 18750 from Samford, southeast Queensland, and pathotype 3 (accession 22269) from *S. guianensis* 'Graham' from Walkamin, North Queensland. A monocondial isolate of the type A accession WRS32 isolated from leaves of *S. scabra* line 10042 from Walkamin, was used for comparison.

The interactions between the three type B pathotypes of *C. gloeosporioides* and the six cultivars or accessions of *S. guianensis* are shown in Table 1.

Maintenance of inoculum and inoculation. Inoculum of the fungus was produced as previously described (6) and standardized at 10^6 spores ml^{-1}. Leaves of 6-wk-old plants were atomized with a suspension of conidia until runoff. The plants were then placed in the dark in a dew chamber at 25 C for 24 hr, before transfer to a controlled environment cabinet with a 14-hr photoperiod, temperature 24–26 C and relative humidity 60–70%.

Sampling. Three leaves of each pathotype/plant combination were sampled at 24, 48, 96, and 192 hr after inoculation. The youngest fully expanded leaves at the time of inoculation were sampled. Whole leaves were stained in alcoholic lactophenol cotton blue and cleared in choral hydrate following the technique of Shipton and Brown (12) except that the leaves were cleared for up to 4–5 days before mounting in 50% glycerol for examination at ×1,000 magnification. Three leaflets per leaf were placed on slides, adaxial surface uppermost, and 100 appressoria examined on each leaflet. Penetration was recorded when a hyphal disk or colony was seen beneath an appressorium. Colony area in epidermal cells was recorded with a calibrated eyepiece grid. The number of epidermal cells infected by the fungus, the number of appressoria associated with a response to the presence of the fungus, and the number of cells responding were also recorded.

Statistical analysis. Angular-transformed penetration data were analyzed using a randomized complete block design with each leaf regarded as a block. Colony area was analyzed similarly using mean colony area $leaflet^{-1}$. Means were compared using least significant differences.

Qualitative study of the compatible interaction between a type B pathotype of *C. gloeosporioides* and *S. guianensis* 'Endeavour'. Three circles (approximately 3 mm in diameter) were drawn on the surface of a number of leaflets of Endeavour. A suspension of conidia (10^6 ml^{-1}) of type B pathotype 1 was placed within each circle using an inoculating loop. At 24, 48, 72, 96, and 120 hr after inoculation, three leaflets were sampled and the pieces of tissue bearing the circles removed. The pieces of tissue were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in a series of graded acetones and embedded in Spurr's low viscosity resin before sectioning at 3 μm on an LKB 8800 Ultratome III (Bromma, Sweden). Sections were stained using the rapid, one-step polychromatic technique described by Alsop (1).

TABLE 1. Interactions between three type B pathotypes of *Colletotrichum gloeosporioides* and six cultivars or accessions of *Stylosanthes guianensis*

	Interaction (C = compatible; I = incompatible)					
	S. guianensis cultivar or line					
Pathotype	Endeavour	34911	Graham	18750	79639	79637
1	C	I	I	I	I	I
2	C	I	I	C	I	I
3	C	I	C	I	I	I

Qualitative comparison of compatible type A and type B infections by *C. gloeosporioides*. *Whole leaves.* Leaves of *S. scabra* 'Fitzroy' were inoculated as previously described with type A conidia. At the same time, leaflets of *S. guianensis* 'Endeavour' were inoculated with conidia of type B pathotype 1. Inoculated leaflets were stained and cleared (12) at 3 wk after inoculation before examination at ×1,000 magnification.

Scanning electron microscopy. Pieces of leaves of *S. guianensis* 'Endeavour' inoculated with type B pathotype 1 conidia and of *S. scabra* 'Fitzroy' inoculated with type A conidia were sampled at 24, 48, 72, 96, and 192 hr after inoculation. The pieces of tissue were exposed to osmium tetroxide vapor overnight, de-

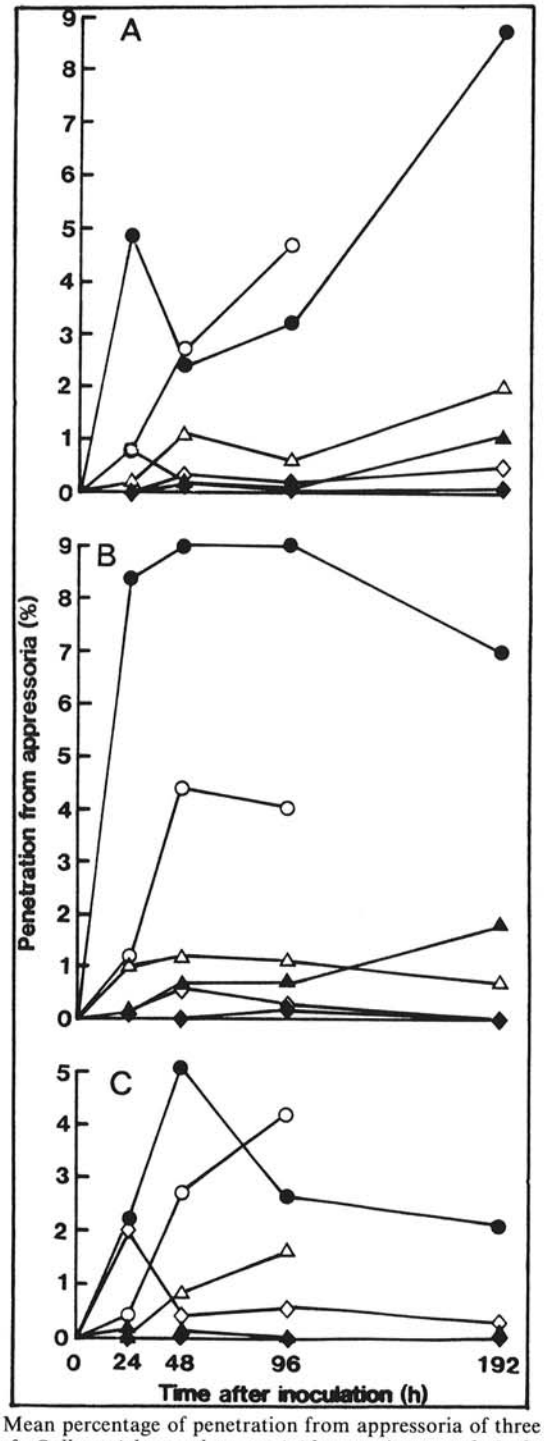

Fig. 1. Mean percentage of penetration from appressoria of three pathotypes of *Colletotrichum gloeosporioides* on leaves of *Stylosanthes guianensis* at four times after inoculation. **A,** Pathotype 1; **B,** Pathotype 2; **C,** Pathotype 3; ○ Endeavour, ● 34911, △ Graham, ▲ 18750, ◇ 79639, ◆ 79637. LSD (5%) = 0.8%.

hydrated in a series of graded acetones, critical point dried, mounted on stubs, and sputter-coated with platinum before viewing on a Philips 505 scanning electron microscope.

RESULTS

Quantitative studies of infection of *S. guianensis* by type B pathotypes of *C. gloeosporioides*. Penetration from appressoria on most host-pathogen combinations commenced by 24 hr after inoculation (Fig. 1). Penetration varied significantly among cultivars ($P < 0.001$), pathotypes ($P < 0.001$), and sampling times ($P < 0.01$). The percentage of penetration from appressoria was very low. The highest level (9%) of penetration was observed in the incompatible 34911-pathotype 2 interaction. For all three pathotypes, penetration occurred more rapidly and more frequently on 34911 than on the other cultivars or accessions. On the susceptible cultivar Endeavour, the percentage of appressoria from which penetration occurred reached a maximum level of 4–5% for all three pathotypes. Penetration from appressoria of pathotypes 1 and 3 on Endeavour continued until at least 96 hr after inoculation. Accurate observations could not be made at 192 hr after inoculation because of extensive growth of secondary hyphae. On Graham, 2% or fewer of appressoria produced vesicles despite Graham-pathotype 3 being a compatible interaction. On 18750, 79639, and 79637, penetration generally occurred from 1% or fewer of appressoria. Penetration was observed from only six of 10,800 appressoria examined on 79637. Overall, penetration occurred more frequently from appressoria that formed over cells of the stomatal complex (546 penetrations) than over other epidermal cells (456 penetrations).

Not all vesicles continued developing to become colonies even in compatible interactions. No colony growth was observed 24 hr after inoculation (Table 2). Growth proceeded relatively slowly between 24 and 48 hr, with colonies of pathotype 1 on even the susceptible cultivar, Endeavour, reaching a mean area of only 420 μm^2. Hyphae were confined to one, occasionally two, cells. Significant differences ($P < 0.001$) in colony areas among cultivars and accessions became apparent after 48 hr. In compatible interactions at 192 hr after inoculation, colonies could not be measured because of the development of an extensive network of secondary "runner" hyphae which spread out from the original infection site and initiated secondary infections. The "runner" hyphae lay above the epidermal cells and were thought to be subcuticular. Secondary infections occurred principally in cells of the stomatal complex. Acervuli were present at this stage.

In incompatible interactions, colonies ceased growth, grew very slowly, or apparently decreased in size. In some cases, the hyphae appeared to be breaking down. Most infections (179 out of 200) were restricted to one cell, although occasionally, up to six cells were infected. "Runner" hyphae were not observed in incompatible interactions.

The response of host cells to infection was recorded only in epidermal cells. Frequently, the host cell beneath an appressorium showed evidence of reacting to the presence of the appressorium, even when penetration from that appressorium could not be observed. Often the cell adjacent to a cell underlying an appressorium appeared to be responding, rather than the cell beneath the appressorium. The host cell response varied from the aggregation of granular cytoplasm beneath an appressorium, through the granular appearance of the entire cytoplasm of the cell with or without the nucleus and nucleolus becoming conspicuous or the cell contents pulling away from the cell wall, to browning of the cell wall and the cell contents. Browning was the least common response. Where browning occurred, it was usually associated with appressoria from which penetration could not be observed, and usually occurred in a cell adjacent to the cell over which the apressorium had formed.

Graham-pathotype 2 was the only interaction showing any response to infection at 24 hr after inoculation. In most interactions both the number of cells responding and the percentage of appressoria around which a response was observed increased with time, especially between 96 and 192 hr. However, in the 34911-pathotype 1, Graham-pathotype 2, and to a lesser extent 79637-pathotype 2 interactions (all incompatible) the percentage of appressoria associated with a response increased rapidly between 24 and 48 hr and more slowly thereafter. In the susceptible cultivar Endeavour, at 48 ahd 96 hr, the contents of many epidermal cells beneath appressoria appeared granular, although there was no evidence of penetration. At 192 hr, extensive necrotic areas were associated with the most extensive colonization.

Qualitative study of the compatible interaction between a type B pathotype of *C. gloeosporioides* and *S. guianensis* 'Endeavour'. A number of vesicles were found in the cuticle or the outer wall of the epidermis (Fig. 2) of leaves of Endeavour infected with type B pathotype 1. In most cases, they were associated with discoloration of the surrounding tissue or deposition of material on the inner surface of the outer wall of the epidermal cell (Fig. 2). No further development was ever observed from infections

TABLE 2. Mean area (μm^2) of colonies of three type B pathotypes of *Colletotrichum gloeosporoides* in leaves of *Stylosanthes guianensis* at three times after inoculation[y]

Pathotype	Cultivar or accession	Mean colony area (μm^2) Time after inoculation (hr) 48	96	120
1	Endeavour	420 a[z]	6,185 a	...
	34911	129 a	231 b	439 a
	Graham	211 a	620 b	303 a
	18750	0 a	4 b	45 a
	79639	175 a	16 b	200 a
	79637	25 a	16 b	13 a
2	Endeavour	315 a	6,419 a	...
	34911	237 a	500 b	396 a
	Graham	68 a	550 b	333 a
	18750	138 a	322 b	...
	79639	0 a	299 b	0 a
	79637	0 a	35 b	0 a
3	Endeavour	273 a	8,055 a	...
	34911	194 a	171 b	173 a
	Graham	175 a	4,138 a	...
	18750	0 a	0 b	3 a
	79639	288 a	114 b	140 a
	79637	0 a	0 b	0 a

[y] Colonies in compatible interactions could not be measured at 192 hr after inoculation because of extensive secondary growth.

[z] Colony area was analyzed with a randomized complete block design with each leaf regarded as a block. Means were compared using least significant differences. Values within each column followed by the same letter did not differ significantly at the 1% level.

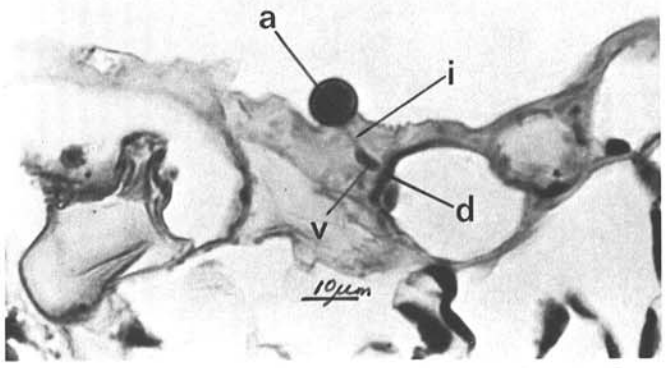

Fig. 2. An appressorium (a), infection peg (i), and vesicle (v) of type B pathotype 1 of *Colletotrichum gloeosporioides* in the cuticle or epidermal cell wall of a leaf of *Stylosanthes guianensis* 'Endeavour' at 48 hr after inoculation. Note the discoloration (d) of the surrounding plant tissue.

such as these. Infections were found in which the infection peg had penetrated the cuticle and the outer wall of the epidermal cell and formed a more or less spherical vesicle within the epidermal cell (Fig. 3). Subsequently, epidermal cells became filled with hyphae (Fig. 4) and inter- and intracellular growth through the palisade mesophyll began (Fig. 4). Hyphae were present in the spongy mesophyll by 72 hr, in parenchyma cells surrounding the vascular bundles by 96 hr, and on the lower epidermis by 120 hr after inoculation.

Sections of later stages of the infection process showed some subcuticular hyphae but in many more sections, considerable amounts of superficial hyphae were present (Fig. 5). Sometimes, hyphae appeared to emerge from infected epidermal cells and grow across the surface of the leaf. Superficial hyphae penetrated host cells without the formation of appressoria (Fig. 5).

Darkened epidermal tissue in response to infection was present as early as 24 hr after inoculation. By 48 hr, the nucleus of the epidermal cell was obvious below the appressorium, and underlying palisade cells were showing signs of disruption and darkening. By 120 hr extensive areas of necrotic tissue were present. Individual infections, even on the one leaf, varied greatly in the amount of colony growth and in the timing and extent of response to infection.

Qualitative comparison of compatible type A and type B infections by *C. gloeosporioides*. *Whole leaves.* The mode of growth of the pathogen corresponded well with the final disease symptoms (limited lesions for type A interactions, blight for type B interactions). In type A infections, a central nonstaining area of tissue contained the developing acervulus. This area was surrounded by a broad (6–10 cells) band of brown, presumably necrotic, cells. The margins of the band were distinctly defined macroscopically and microscopically. Fungal hyphae were observed outside the band of necrotic tissue only once, and then only a single, short (approximately 10 μm) hypha. In type B infections, necrotic tissue had no distinct margin, "runner" hyphae extended over apparently healthy cells and initiated secondary infections.

Scanning electron microscopy. By 72 hr after inoculation, an extensive network of "runner" hyphae was present on the surface of leaves of Endeavour inoculated with the type B pathogen (Fig. 6). No such hyphae were present on leaves of Fitzroy inoculated with the type A pathogen. On Endeavour, hyphae sometimes appeared to emerge through the epidermis and grow across the surface of the leaf (Fig. 7). Other hyphae appeared to emerge from appressoria before growing over the leaf surface (Fig. 7).

DISCUSSION

This paper reports for the first time quantitative histological studies of compatible and incompatible interactions between genetically defined cultivars and accessions of *S. guianensis* and type B pathotypes of *C. gloeosporioides*. Penetration had commenced on most cultivars and accessions by 24 hr after inoculation. This is in agreement with results reported for a number of *Colletotrichum* spp. on a range of hosts (10,11,16,17) although TeBeest et al (15) reported no infection before 48 hr after inoculation. Penetration on some cultivars or accessions continued until 96 hr. Trevorrow et al (16) reported similar results with type A isolates of *C. gloeosporioides* on *S. scabra* while Skipp and Deverall (13) found that penetration from appressoria of *C. lindemuthianum* (Sacc. & Magn.) Br. & Cav. on bean (*Phaseolus vulgaris* L.) hypocotyls continued until 66–74 hr after inoculation. Skipp and Deverall (13) and Trevorrow et al (16) reported marked variations in penetration among sampling times—a phenomenon also observed in these experiments.

Results reported here confirm previous reports (10,16) that penetration of *Stylosanthes* leaves by *C. gloeosporioides* occurred

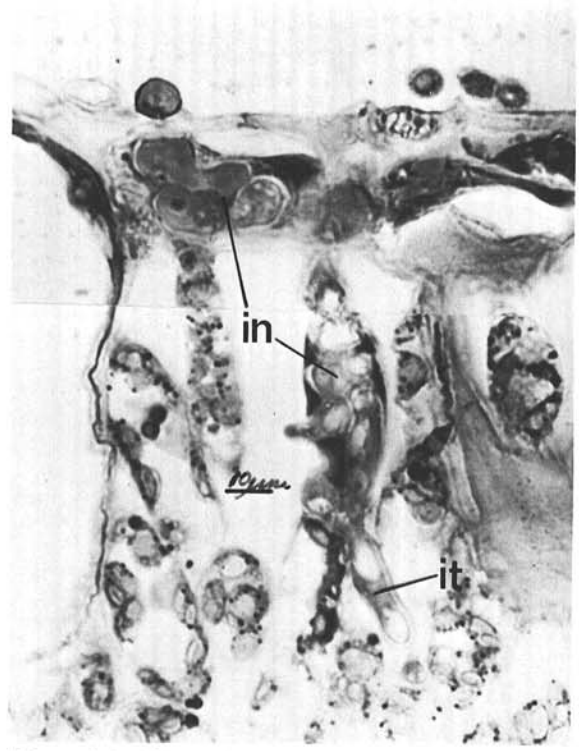

Fig. 4. Intracellular (in) and intercellular (it) growth of type B pathotype 1 of *Colletotrichum gloeosporioides* in a leaf of *Stylosanthes guianensis* 'Endeavour' at 96 hr after inoculation.

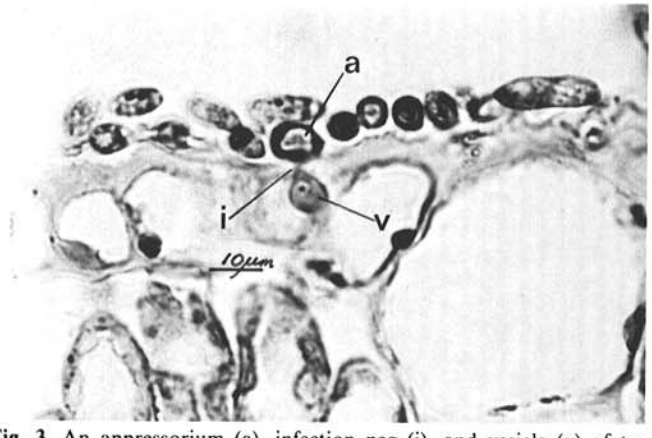

Fig. 3. An appressorium (a), infection peg (i), and vesicle (v) of type B pathotype 1 of *Colletotrichum gloeosporioides* within an epidermal cell of a leaf of *Stylosanthes guinanesis* 'Endeavour' at 72 hr after inoculation.

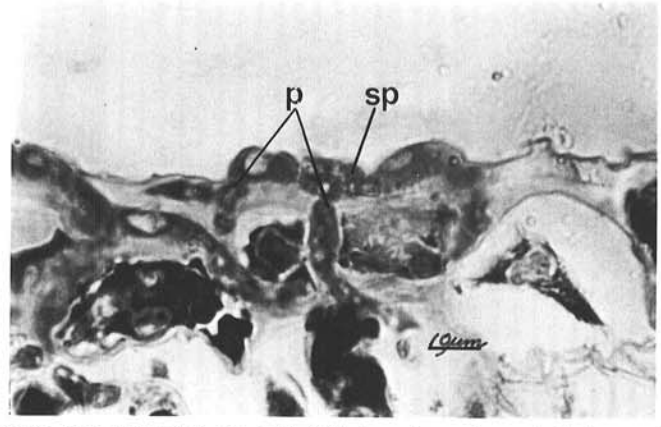

Fig. 5. Penetration (p) of a leaf of *Stylosanthes guianensis* 'Endeavour' at 120 hr after inoculation, by a superficial hypha (sp) of type B pathotype 1 of *Colletotrichum gloeosporioides* without the formation of an appressorium.

more frequently through cells of the stomatal complex than through epidermal cells. Penetration through the stomatal aperture was not observed.

One of the most conspicuous features of the results was the low level (9% or less) of penetration from appressoria. Ogle et al (10) and Vinijsanun et al (17) reported similarly low levels of penetration of type B isolates of *C. gloeosporioides* on *S. guianensis* 'Endeavour' (susceptible). With type A isolates, penetration occurred from up to approximately 37% of appressoria (16). However, TeBeest et al (15) found that by 72 hr after inoculation, penetration had occurred from only about 10% of appressoria of *C. gloeosporioides* on *Aeschynomene virginica* (L.) BSP. They suggested that the low level of penetration may be due to unfavorable incubation temperatures as Skoropad (14) had found that penetration occurs over a much narrower range of temperatures than appressorium formation. Higher levels of penetration were not related to compatible interactions as reported for type A interactions of *C. gloeosporioides* on *S. scabra* (16). In fact, highest levels of penetration, as well as most rapid penetration, was observed in incompatible interactions with 34911. Lower penetration was recorded on interactions involving 79637, which contains three resistance genes, than for any of the other interactions studied. This result suggests that resistance in this accession could be manifested by a different mechanism(s) from that in the other genotypes.

The production of an infection vesicle or knot in the cuticle of *S. guianensis* 'Endeavour' by type B isolates of *C. gloeosporioides* has been previously reported (17) and was thought to represent the first stage in the infection process. Although a number of such vesicles were found in this study, no further development was observed from them. We suggest that such swellings are examples of unsuccessful infection attempts, especially since they were frequently accompanied by discoloration (also reported by Vinijsanun et al [17]) and/or deposition of apposition layers on the inner surface of the outer wall of the epidermal cell similar to that described in unsuccessful infections of *C. gloeosporioides* on mature leaves of *Populus tremuloides* Michx. (8). For successful infection to occur, it appears that the infection peg must grow through the cuticle and the epidermal cell wall to form a vesicle within an epidermal cell, as in type A infections (16).

Slow growth of colonies over the first 48 hr after inoculation, to the stage where up to two to three cells were infected has also been reported for *C. gloeosporioides* on *P. tremuloides* (8). In compatible type B *C. gloeosporioides-S. guianensis* interactions, subsequent development is rapid. Secondary hyphae grow inter- and intracellularly through the mesophyll and also superficially across the surface of the leaf. Previously (10), rapid lateral spread of colonies had been attributed to subcuticular hyphal growth. This study showed that, while subcuticular hyphae are present, superficial growth is much more common. Moreover, superficial hyphae effect penetration without the formation of appressoria. Penetration of alfalfa, *Medicago sativa* L., stems by hyphal strands of *Colletotrichum trifolii* Bain & Essary without formation of appressoria was recently reported (11), while TeBeest et al (15) reported that *C. gloeosporioides* infected stems of *A. virginica* most rapidly through trichomes without the formation of appressoria. Superficial hyphae have not been reported in type A infections of *C. gloeosporioides* (16).

Differences in colony size between compatible and incompatible interactions were apparent by 48 hr after inoculation in type A infections (16), but not until after 48 hr in the type B interactions studied here. This observation is consistent with earlier reports (7) that type A infections develop more rapidly than type B infections. In incompatible type A interactions, colony area in most interactions did not increase significantly beyond 48 or 96 hr after inoculation and although the maximum number of cells infected in any incompatible interaction was six, the majority of infections were contained within a single cell (16). Similarly, in *C. lindemuthianum* infections on bean, infections were frequently confined to one cell or to a small group of cells (9). No secondary hyphae developed. Results reported in this paper are consistent with these observations. The apparent decrease in colony size in some incompatible interactions could be due to the breakdown of hyphae or to the disruption of host cells making accurate measurements difficult.

In type A infections, cell degradation was not evident with light microscopy in the first 48 hr after inoculation (16). Collapse of epidermal and mesophyll cells in susceptible hosts was evident by 72 hr. However, in compatible type B interactions, no cell collapse was observed during the first 72 hr after inoculation (17). In these studies, cell degradation was evident in only one interaction (incompatible) before 48 hr after inoculation and by 72 hr epidermal and mesophyll cell collapse was evident in some infections.

It appears that the infection process by type B isolates of *C. gloeosporioides* on *S. guianensis* is as follows. Spores on the surface of the leaf germinate and form appressoria from which fine infection pegs grow through the cuticle and the outer wall of the epidermal cells to form vesicles within the epidermal cells. A small group of epidermal cells usually becomes filled with hyphae before secondary intra- and intercellular growth occurs. At the same time, other secondary hyphae emerge through the epidermis or from the appressoria and grow across the surface of the leaf, penetrating without the formation of appressoria and initiating new infections, principally in the cells of stomatal complexes. This is the first time this mode of infection has been reported for this host-pathogen combination. In incompatible interactions, the infection process is similar, but most infections

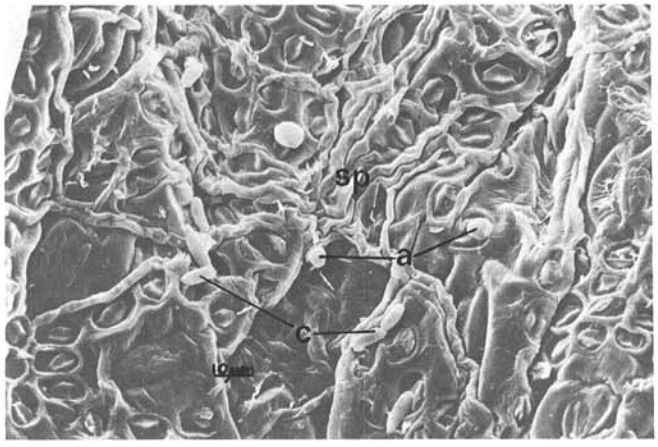

Fig. 6. Conidia (c), appressoria (a), and network of superficial hyphae (sp) of type B pathotype 1 of *Colletotrichum gloeosporioides* on the surface of a leaf of *Stylosanthes guianensis* 'Endeavour' at 72 hr after inoculation.

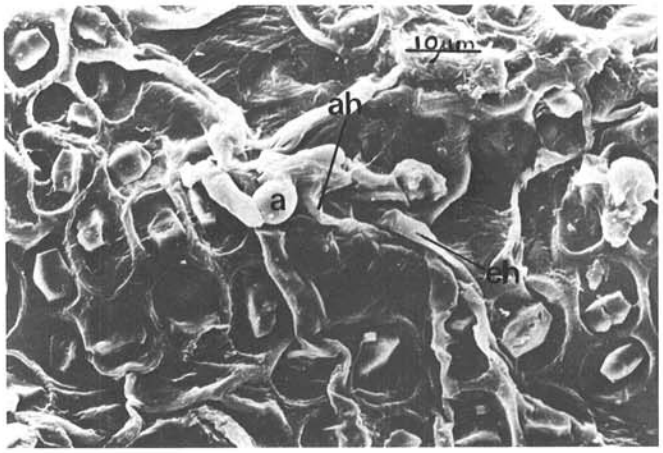

Fig. 7. Superficial hyphae of type B pathotype 1 of *Colletotrichum gloeosporioides* on the surface of a leaf of *Stylosanthes guianensis* 'Endeavour' at 72 hr after inoculation. Some hyphae (ah) appear to emerge from the appressorium (a), others (eh) from beneath the epidermis.

are confined to one epidermal cell. Intracellular, intercellular, subcuticular, and superficial secondary growth does not occur. Response of host epidermal cells to the presence of the fungus varies from granulation of the cytoplasm to browning of the cell wall and contents.

These studies have allowed us to establish the timing of these events in compatible and incompatible interactions, and work is now being undertaken to determine host biochemical responses that may be related to the restriction of fungal growth in incompatible interactions.

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